Effect of water activity and humectant identity on the growth kinetics of *Escherichia coli* O157:H7

The effect of three non-ionic humectants on the growth kinetics of a three-strain mixture of Escherichia coli *O157:H7 was studied in brain–heart infusion broth. A full factorial design was used to test mannitol (0, 50, 100, 150, 200 g l^{-1}), sorbitol (0, 50, 10, 150, 200 g l^{-1}), and sucrose (0, 50, 100, 200, 300 g l^{-1}) in combination with four pH levels (4·5, 5·5, 6·5, 7·5) and three incubation temperatures (12°, 19°, and 28°C). Growth was measured using viable counts, and growth curves were then fitted using the Gompertz equation to derive generation times, lag phase durations, maximum population densities, and 'times to a 1000-fold increase'. Increasing humectant concentrations (i.e., lowering water activity (a_w)) interacted with decreasing temperatures and pH values to increase lag phase durations and generation times. The results were compared with an earlier study where NaCl was used as the humectant. The response surface model developed for NaCl in the earlier study was evaluated for its ability to successfully predict the growth kinetics of* E. coli *observed with the three non-ionic solutes. The model provided reasonable estimates for all four humectants, particularly at higher a_w values. As the minimum a_w that supported growth was approached, differences among the solutes were observed and the model was less accurate. This was evidenced by conservative (fail–safe) predictions that over estimated the pathogen's growth potential at low a_w values. Overall, the results indicate that models developed using NaCl as a humectant can be used to obtain estimates of a bacterium's growth in the presence of other solutes.*

Introduction

During the past several years, research in predictive food microbiology has provided a wealth of quantitative data on the impact that various intrinsic and extrinsic parameters have on the growth of foodborne pathogenic bacteria. Water activity (a_w), temperature, and pH have been identified as the three primary factors controlling microbial growth in many foods. However, most investigators have not directly considered a_w. Instead, they have modeled the effect of sodium chloride concentration, the humectant used to modify a_w (McMeekin et al. 1987, Gibson et al. 1988, Buchanan and Phillips 1990, Palumbo et al. 1991, 1992, Zaika et al. 1992, 1994, Quintavalla and Parolari 1993, Bhaduri et al. 1994, 1995, Buchanan and Bagi 1994, Sutherland and Bayliss 1994, Sutherland et al. 1994, 1995). In many

Table 1 (continued). Effect of mannitol concentration, initial pH, and incubation temperature on the growth of *Escherichia coli* O157:H7 in brain–heart infusion broth

					EGR		GT		LPD		MPD		T-1000	
TEM	pH	Man	Aw	*n*	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
12	5·5	0	0·987	2	0·087	0·028	3·9	1·3	22·2	2·5	10·0	<0·1	60·9	15·1
12	5·5	50	0·982	4	0·055	0·004	5·5	0·4	41·7	4·1	9·8	0·1	97·5	7·9
12	5·5	100	0·976	5	0·030	0·015	13·2	6·0	56·4	51·1	9·4	0·7	188·6	72·5
12	5·5	150	0·972	6	0·021	0·007	17·2	11·4	75·2	41·5	7·0	2·0	209·2	*
12	5·5	200	0·968		NT-BLS									
12	6·5	0	0·987	2	0·106	0·035	3·2	1·0	17·2	1·5	9·8	<0·1	48·9	11·9
12	6·5	50	0·982	3	0·065	0·058	9·0	4·9	40·2	47·4	9·5	0·2	130·6	2·0
12	6·5	100	0·976	2	0·096	0·018	3·3	0·6	104·3	5·3	9·7	0·1	137·0	1·0
12	6·5	150	0·972	2	0·037	0·004	8·3	1·0	63·9	23·9	9·1	<0·1	147·1	13·8
12	6·5	200	0·968		NT-BLS									
12	7·5	0	0·987	2	0·134	0·003	2·2	<0·1	18·8	0·7	9·7	<0·1	41·2	0·2
12	7·5	50	0·982	2	0·059	<0·001	5·1	<0·1	25·8	1·1	9·7	<0·1	78·0	0·8
12	7·5	100	0·976	4	0·046	0·019	7·8	3·2	79·2	30·5	9·7	0·3	157·4	2·5
12	7·5	150	0·972		NT-BLS									
12	7·5	200	0·968		NT-BLS									

*Not all cultures increased 1000-fold. Value based on the average of the six cultures. Tem=temperature (C); Man=mannitol (gl^{-1}); a_w=water activity; *n*=number of replicates; EGR=exponential growth rate [(log (cfu ml^{-1})) h^{-1}]; GT=generation time [h]; LPD=lag phase duration [h]; MPD=maximum population density [log (cfu ml^{-1})]; T_{1000}=time to 1000-fold increase in population density [h]; s.d.=standard deviation; NT=not tested; NT-BLS=not tested, beyond limit of solubility; NG=no growth.

250 ml Erlenmeyer flasks, which were sealed with foam plugs, and autoclaved for 15 min at 121°C. The pH of the media were verified periodically to remain within 0·1 pH units after autoclaving. The flasks were inoculated with 0·5 ml of the diluted mixture of the three *E. coli* strains to achieve an initial level of approximately 10^3 cfu ml^{-1}. The flasks were incubated with agitation (150 rm^{-1}) at 12°, 19°, or 28°C. Periodically, 2·5 ml samples were removed, diluted as needed using 0·1% peptone water, and surface plated in duplicate on BHI agar using a Spiral Plater (Spiral Systems, Inc., Bethesda, MD, USA). All plates were incubated at 37°C for 20–24 h and then enumerated using an automatic colony counter (Spiral Systems, Inc.).

Growth curves

Viable counts were converted to Log_{10} values, and growth curves generated by fitting the data to the Gompertz equation as described previously (Gibson et al. 1988, Buchanan et al. 1989). The Gompertz parameters were then used to calculate lag phase duration (LPD), exponential growth rate (EGR), generation time (GT), maximum population density (MPD), and time to 1000-fold increase in population density (T_{1000}) as described previously (Buchanan et al. 1989).

A_w determinations

The relationship between humectant concentration and a_w in BHI was determined by supplementing the medium in 5 g l^{-1} increments with crystalline NaCl (5–55 g l^{-1}), in 20 g l^{-1} increments with sorbitol and mannitol (0–220 g l^{-1}), or 30 g l^{-1} increments with sucrose (0–330 g l^{-1}). The a_w was determined at 24–25°C using a water activity meter (Aqua Lab model CX-2; Decagon Devices Inc., Pullman, WA, USA). All determinations were done at least twice and the results were analysed by linear regression. Unsupplemented BHI has a basal level of 5 g l^{-1} NaCl, and had a measured a_w of 0·987 with a standard deviation of 0·0009 (*n*=12). The effect of added humectant (gl^{-1}) on the a_w of BHI could then be estimated using the equations:

Table 2. Effect of sorbitol concentration, initial pH, and incubation temperature on the growth of *Escherichia coli* O157:H7 in brain–heart infusion broth. (See Table 1 for abbreviations)

					EGR		GT		LPD		MPD		T-1000	
TEM	pH	Sorb	Aw	*n*	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
28	4·5	0	0·987	3	0·418	0·045	0·7	0·1	10·4	5·8	9·5	0·2	17·7	6·0
28	4·5	50	0·983	3	0·291	0·093	1·1	0·3	19·1	0·6	9·2	0·3	30·4	3·6
28	4·5	100	0·978	2	0·282	0·004	1·1	<0·1	18·4	0·1	10·1	0·1	29·2	0·1
28	4·5	150	0·974	2	0·000	—	NG		—		—		—	
28	4·5	200	0·969		NT									
28	5·5	0	0·987	2	0·553	0·002	0·5	<0·1	2·3	0·4	9·7	0·2	7·7	0·6
28	5·5	50	0·983	2	0·444	0·009	0·7	<0·1	2·7	0·1	9·0	<0·1	9·6	<0·1
28	5·5	100	0·978	2	0·362	0·004	0·8	<0·1	2·6	<0·1	9·0	<0·1	11·1	0·2
28	5·5	150	0·974	2	0·452	0·024	0·7	<0·1	5·2	0·4	9·4	<0·1	12·2	0·3
28	5·5	200	0·969	2	0·263	0·005	1·1	<0·1	7·1	0·2	9·2	<0·1	18·7	0·5
28	6·5	0	0·987	3	0·548	0·014	0·5	<0·1	3·0	0·1	9·7	<0·1	8·4	0·1
28	6·5	50	0·983	2	0·483	0·050	0·6	0·1	3·1	0·5	10·6	0·1	9·4	1·2
28	6·5	100	0·978	2	0·419	0·069	0·7	0·1	4·4	0·3	9·2	0·1	11·9	0·9
28	6·5	150	0·974	2	0·369	0·007	0·8	<0·1	3·1	0·3	9·2	<0·1	11·3	0·4
28	6·5	200	0·969	2	0·316	0·004	1·0	<0·1	3·9	0·1	9·4	<0·1	13·5	0·3
28	7·5	0	0·987	3	0·537	0·004	0·6	<0·1	2·8	0·1	9·4	<0·1	8·4	0·1
28	7·5	50	0·983	2	0·477	0·021	0·6	<0·1	3·3	0·7	10·3	0·3	9·6	1·0
28	7·5	100	0·978	2	0·501	0·049	0·6	0·1	4·9	0·6	9·4	<0·1	11·0	<0·1
28	7·5	150	0·974	2	0·388	0·026	0·8	<0·1	3·4	0·4	9·2	<0·1	11·3	0·1
28	7·5	200	0·969	2	0·266	0·010	1·1	<0·1	4·5	0·0	9·6	0·4	15·9	0·4
19	4·5	0	0·987	3	0·120	0·006	2·5	0·1	17·5	2·0	10·1	0·1	42·6	1·1
19	4·5	50	0·983	2	0·000	—	NG		—		—		—	
19	4·5	100	0·978		NT									
19	4·5	150	0·974		NT									
19	4·5	200	0·969		NT									
19	5·5	0	0·987	3	0·256	0·031	1·2	0·2	12·2	1·4	10·2	0·3	24·1	0·2
19	5·5	50	0·983	2	0·220	0·003	1·4	<0·1	15·4	<0·1	8·9	0·1	29·1	0·2
19	5·5	100	0·978	2	0·135	0·003	2·2	<0·1	17·4	0·1	9·6	<0·1	39·7	0·6
19	5·5	150	0·974	2	0·109	0·005	2·8	0·1	22·9	0·4	9·2	<0·1	50·8	0·9
19	5·5	200	0·969	2	0·055	0·009	5·6	0·9	26·1	0·3	8·7	0·2	83·2	9·7
19	6·5	0	0·987	3	0·247	0·005	1·2	<0·1	9·7	0·2	9·7	<0·1	21·9	0·3
19	6·5	50	0·983	2	0·155	0·012	2·0	0·1	11·2	0·8	10·3	0·1	30·7	0·7
19	6·5	100	0·978	2	0·173	0·001	1·7	<0·1	16·9	0·3	9·7	0·2	34·3	0·2
19	6·5	150	0·974	2	0·119	0·007	2·5	0·1	16·6	0·5	9·3	<0·1	42·0	0·9
19	6·5	200	0·969	2	0·052	0·003	6·0	0·4	48·2	0·6	9·3	<0·1	107·8	3·6
19	7·5	0	0·987	3	0·261	0·016	1·2	0·1	9·2	0·9	9·6	0·1	20·8	0·3
19	7·5	50	0·983	2	0·197	0·018	1·5	0·1	16·3	0·5	10·0	0·1	31·6	0·9
19	7·5	100	0·978	2	0·123	0·003	2·5	0·1	19·4	0·7	9·6	<0·1	44·0	0·1
19	7·5	150	0·974	2	0·108	0·003	2·8	0·1	23·8	2·7	9·5	0·1	51·5	1·8
19	7·5	200	0·969	2	0·068	0·010	4·6	0·7	83·8	5·3	9·3	<0·1	129·2	11·8
12	4·5	0	0·987	1	0·000	—	NG		—		—		—	
12	4·5	50	0·983	2	0·000	—	NG							
12	4·5	100	0·978		NT									
12	4·5	150	0·974		NT									
12	4·5	200	0·969		NT									

Table 2 (continued). Effect of sorbitol concentration, initial pH, and incubation temperature on the growth of *Escherichia coli* O157:H7 in brain–heart infusion broth. (See Table 1 for abbreviations)

					EGR		GT		LPD		MPD		T-1000	
TEM	pH	Sorb	Aw	*n*	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
12	5·5	0	0·987	2	0·087	0·028	3·9	1·3	22·2	2·5	10·0	<0·1	60·9	15·1
12	5·5	50	0·983	4	0·052	0·003	5·8	0·3	32·7	11·1	9·7	0·1	92·0	14·4
12	5·5	100	0·978	2	0·014	<0·001	21·5	0·3	29·1	3·5	8·6	<0·1	249·7	1·2
12	5·5	150	0·974	4*	0·017	<0·001	17·6	<0·1	39·2	4·5	4·9	<0·1	—	
12	5·5	200	0·969	2	0·000	—	NG		—		—		—	
12	6·5	0	0·987	2	0·106	0·035	3·2	1·0	17·2	1·5	9·8	<0·1	48·9	11·9
12	6·5	50	0·983	2	0·054	0·001	5·6	0·1	21·6	0·4	9·6	<0·1	77·4	0·6
12	6·5	100	0·978	1	0·047	—	6·41	—	31·9	—	9·6	—	95·9	—
12	6·5	150	0·974	2	0·016	0·001	18·7	1·3	156·6	12·3	8·1	0·3	362·2	18·6
12	6·5	200	0·969	2	0·000	—	NG		—		—		—	
12	7·5	0	0·987	2	0·134	0·003	2·2	<0·1	18·8	0·7	9·7	<0·1	41·2	0·2
12	7·5	50	0·983	2	0·076	0·007	4·0	0·4	56·3	6·3	9·6	<0·1	96·3	2·7
12	7·5	100	0·978	6	0·037	0·007	8·5	1·9	145·2	78·9	9·6	0·3	229·8	77·0
12	7·5	150	0·974	2	0·018	0·001	16·7	1·0	46·5	3·9	8·4	0·3	216·5	12·6
12	7·5	200	0·969	2	0·000	—	NG		—		—		—	

*Only two of the four cultures grew. The values indicated are the averages of the two that grew. The two cultures that grew did not achieve a 1000-fold increase in population density.

Sodium Chloride (added):

$$a_w = -0{\cdot}000496 \times NaCl + 0{\cdot}987$$
$$R^2 = 0{\cdot}991$$

Mannitol:

$$a_w = -0{\cdot}000102 \times Mannitol + 0{\cdot}987$$
$$R^2 = 0{\cdot}982$$

Sorbitol:

$$a_w = -0{\cdot}000089 \times Sorbitol + 0{\cdot}987$$
$$R^2 = 0{\cdot}984$$

Sucrose:

$$a_w = -0{\cdot}000049 \times Sucrose + 0{\cdot}987$$
$$R^2 = 0{\cdot}975$$

The relative impact of humectant concentration on a_w for the four humectants is depicted graphically in Fig. 1.

Due to its solubility limits at this temperature, the linear regression for mannitol only included the concentration range of 0–180 g l^{-1}. A a_w of 0·680 was assumed for the 200 g l^{-1} mannitol samples.

Results

The effects of mannitol, sorbitol and sucrose supplementation on the growth kinetics of *E. coli* O157:H7 are summarized in Tables 1–3, respectively. The experimental values listed for BHI without humectant supplementation were largely acquired during earlier studies (Buchanan and Klawitter 1992, Buchanan et al. 1993, Buchanan and Bagi 1994). Increasing humectant concentrations affected the LPD and GT, but generally had little impact on the MPD reached by the cultures. The few exceptions were typically associated with conditions where there were multiple variables that were limiting (e.g. mannitol, 12°C, pH 5·5 (Table 1)). In general, GTs and LPDs increased proportionally as a_w declined; however, in a few cases (e.g. mannitol/12°C/pH 6·5, sorbitol/12°C/pH 7·5) extended lag phases with relatively rapid generation times were observed. Such growth behavior has been observed commonly with occasional cultures grown under non-optimal growth conditions, which accounts in part for the emergence of statistics such as T_{1000} for evaluating the efficacy of predictive models. The T_{1000} value provides an integrated esti-

Table 3. Effect of sucrose concentration, initial pH, and incubation temperature on the growth of *Escherichia coli* O157:H7 in brain–heart infusion broth. (See Table 1 for abbreviations)

					EGR		GT		LPD		MPD		T-1000	
TEM	pH	Suc	Aw	*n*	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
28	4·5	0	0·987	3	0·418	0·045	0·7	0·1	10·4	5·8	9·5	0·2	17·7	6·0
28	4·5	50	0·985	2	0·350	0·011	0·9	<0·1	4·3	0·4	8·8	<0·1	12·9	0·1
28	4·5	100	0·982	2	0·175	0·002	1·7	<0·1	7·1	0·3	9·3	<0·1	24·3	0·1
28	4·5	200	0·977	2	0·156	0·012	2·0	0·2	13·7	0·6	9·2	0·1	33·2	0·9
28	4·5	300	0·972	2	0·000	—	NG		—		—		—	
28	5·5	0	0·987	2	0·553	0·002	0·5	<0·1	2·3	0·4	9·7	0·2	7·7	0·6
28	5·5	50	0·985	2	0·476	0·003	0·6	<0·1	2·4	<0·1	9·2	<0·1	8·8	0·1
28	5·5	100	0·982	2	0·454	0·008	0·7	<0·1	2·8	<0·1	9·2	<0·1	9·5	0·1
28	5·5	200	0·977	2	0·484	0·014	0·6	<0·1	4·0	0·3	8·7	<0·1	10·3	0·1
28	5·5	300	0·972	2	0·258	0·004	1·2	<0·1	5·2	0·1	9·3	<0·1	16·8	0·1
28	6·5	0	0·987	3	0·548	0·014	0·5	<0·1	3·0	0·1	9·7	<0·1	8·4	0·1
28	6·5	50	0·985	2	0·501	0·005	0·6	<0·1	2·4	<0·1	9·3	<0·1	8·4	<0·1
28	6·5	100	0·982	2	0·498	0·026	0·6	<0·1	3·0	0·3	9·3	0·1	9·1	0·6
28	6·5	150	0·980	2	0·323	0·001	0·9	<0·1	2·2	<0·1	9·3	<0·1	11·6	0·1
28	6·5	200	0·977	2	0·345	0·003	0·9	<0·1	4·6	0·2	9·2	<0·1	13·5	0·2
28	6·5	300	0·972	4	0·403	0·081	0·8	0·2	11·3	5·3	9·1	0·1	19·1	3·8
28	7·5	0	0·987	3	0·537	0·004	0·6	<0·1	2·8	0·1	9·4	<0·1	8·4	0·1
28	7·5	50	0·985	2	0·524	0·013	0·6	<0·1	2·4	<0·1	9·7	<0·1	8·2	0·1
28	7·5	100	0·982	2	0·493	0·015	0·6	<0·1	2·8	0·8	9·7	<0·1	8·9	0·6
28	7·5	200	0·977	2	0·399	0·003	0·8	<0·1	3·9	0·4	9·5	<0·1	11·5	0·5
28	7·5	300	0·972	2	0·267	0·002	1·1	<0·1	5·9	0·3	9·2	0·1	17·3	0·2
19	4·5	0	0·987	3	0·120	0·006	2·5	0·1	17·5	2·0	10·1	0·1	42·6	1·1
19	4·5	50	0·985	2	0·213	0·021	1·4	0·1	11·3	1·6	9·7	0·1	25·7	0·1
19	4·5	100	0·982	2	0·206	0·012	1·5	0·1	15·5	2·7	9·7	0·1	30·2	1·8
19	4·5	200	0·977	2	0·000	—	NG		—		—		—	
19	4·5	300	0·972	2	0·000	—	NG		—		—		—	
19	5·5	0	0·987	3	0·256	0·031	1·2	0·2	12·2	1·4	10·2	0·3	24·1	0·2
19	5·5	50	0·985	2	0·227	0·011	1·3	0·1	12·1	1·0	9·4	<0·1	25·4	0·3
19	5·5	100	0·982	2	0·147	0·004	2·1	0·1	12·8	1·2	9·4	<0·1	33·2	0·6
19	5·5	200	0·977	2	0·140	0·004	2·2	0·1	20·5	0·4	9·3	<0·1	42·0	0·2
19	5·5	300	0·972	2	0·041	0·002	7·4	0·4	156·2	31·9	8·0	0·4	232·0	36·1
19	6·5	0	0·987	3	0·247	0·005	1·2	<0·1	9·7	0·2	9·7	<0·1	21·9	0·3
19	6·5	50	0·985	2	0·270	0·002	1·1	<0·1	17·4	<0·1	9·4	<0·1	28·5	0·1
19	6·5	100	0·982	2	0·146	0·001	2·1	<0·1	14·7	0·2	9·7	<0·1	35·2	0·3
19	6·5	200	0·977	2	0·096	0·001	3·2	<0·1	13·3	0·3	9·0	<0·1	44·9	0·1
19	6·5	300	0·972	2	0·029	0·003	10·6	1·0	56·3	3·2	8·9	0·3	162·0	7·3
19	7·5	0	0·987	3	0·261	0·016	1·2	0·1	9·2	0·9	9·6	0·1	20·8	0·3
19	7·5	50	0·985	2	0·217	0·001	1·4	<0·1	17·4	0·1	9·7	<0·1	31·2	<0·1
19	7·5	100	0·982	2	0·179	0·002	1·7	<0·1	18·8	0·0	9·6	0·1	35·5	0·2
19	7·5	200	0·977	2	0·083	0·002	3·6	0·1	22·2	0·4	9·0	0·1	58·5	0·4
19	7·5	300	0·972	2	0·016	0·003	19·0	3·6	75·4	12·2	8·3	0·7	269·0	26·9
12	4·5	0	0·987	1	0·000	—	NG		—		—		—	
12	4·5	50	0·985	2	0·000	—	NG		—		—		—	
12	4·5	100	0·982	2	0·000	—	NG		—		—		—	
12	4·5	200	0·977		NT									
12	4·5	300	0·972		NT									

Table 3 (continued). Effect of sucrose concentration, initial pH, and incubation temperature on the growth of *Escherichia coli* O157:H7 in brain–heart infusion broth. (See Table 1 for abbreviations)

					EGR		GT		LPD		MPD		T-1000	
TEM	pH	Suc	Aw	*n*	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
12	5·5	0	0·987	2	0·087	0·028	3·9	1·3	22·2	2·5	10·0	<0·1	60·9	15·1
12	5·5	50	0·985	2	0·078	0·001	3·9	0·1	31·7	1·6	9·7	0·2	70·1	2·2
12	5·5	100	0·982	2	0·069	0·009	4·4	0·6	39·4	4·3	9·4	0·2	83·5	10·3
12	5·5	200	0·977	2	0·026	0·002	11·9	0·8	65·3	9·1	9·6	<0·1	184·3	0·7
12	5·5	300	0·972	2	0·000	—	NG		—		—		—	
12	6·5	0	0·987	2	0·106	0·035	3·2	1·0	17·2	1·5	9·8	<0·1	48·9	11·9
12	6·5	50	0·985	2	0·061	<0·001	5·0	<0·1	25·0	0·8	10·6	<0·1	74·5	0·5
12	6·5	100	0·982	2	0·064	<0·001	4·7	<0·1	32·9	1·1	9·6	<0·1	79·5	1·1
12	6·5	200	0·977	2	0·040	<0·001	7·6	0·1	131·7	0·2	9·2	0·1	183·0	25·5
12	6·5	300	0·972	2	0·000	—	NG		—		—		—	
12	7·5	0	0·987	2	0·134	0·003	2·2	<0·1	18·8	0·7	9·7	<0·1	41·2	0·2
12	7·5	50	0·985	2	0·069	0·004	4·4	0·3	44·8	11·6	9·3	0·4	88·9	9·2
12	7·5	100	0·982	2	0·066	0·001	4·6	0·1	44·9	2·5	9·6	<0·1	90·9	1·7
12	7·5	200	0·977	2	0·046	0·001	6·6	0·1	40·6	0·5	9·4	<0·1	106·2	0·3
12	7·5	300	0·972	2	0·000	—	NG		—		—		—	

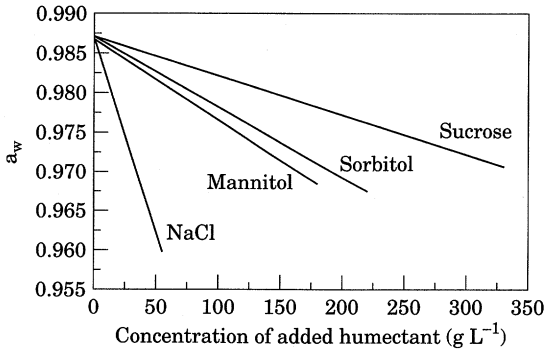

Figure 1. Effect of added mannitol, sorbitol, sucrose, and sodium chloride concentration on the water activity of BHI broth at 24–25°C.

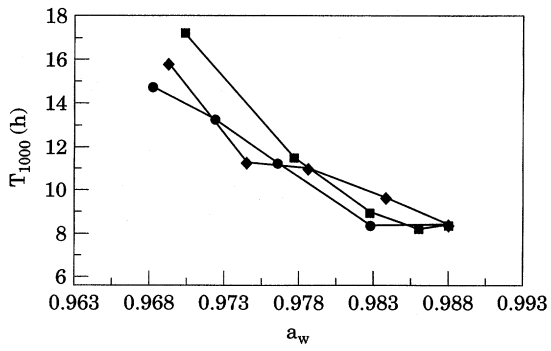

Figure 2. Example of the effect of humectant identity on the time for *E. coli* O157:H7 to achieve a 1000-fold increase in population density. (Culture conditions were 28°C and pH 7·5.) (●) mannitol, (◆) sorbitol, (■) sucrose.

mate of how the initiation and subsequent growth of a micro-organism are affected by cultural conditions. The use of a 3-log increase in population density was based on the selection of an end point that provides a balanced measure of the impact of the lag and exponential growth phases. It is important to note that the general observation and relationships discussed below for T_{1000} values are also pertinent for LPD and GT when these growth kinetics were considered individually.

Comparison of the three humectants suggested that at higher a_w levels, particularly when temperature and pH were non-limiting, the differences between the humectants were minimal (Fig. 2). However, as the environment was made more inhospitable, apparent differences due to humectant identity were noted. While it is difficult to assess quantitatively due to the increased variability of the micro-organism's growth kinetics as the conditions become more limiting, it appears qualitatively that the relative ability of high

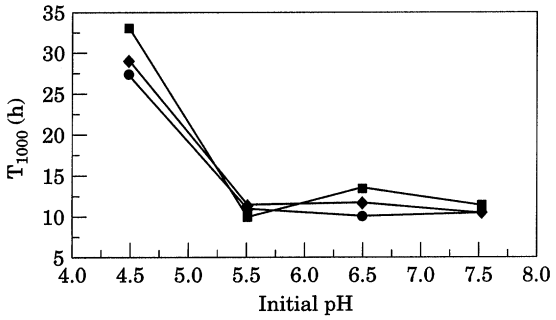

Figure 3. Example of the effect of initial pH on the time for *E. coli* O157:H7 to achieve a 1000-fold increase in population density at 28°C when cultured in BHI supplemented with different humectants. The humectant concentrations were (●) 100 g l^{-1} mannitol (a_w=0·976), (◆) 100 g l^{-1} sorbitol (a_w=0·978), and (■) 200 g l^{-1} (a_w=0·977).

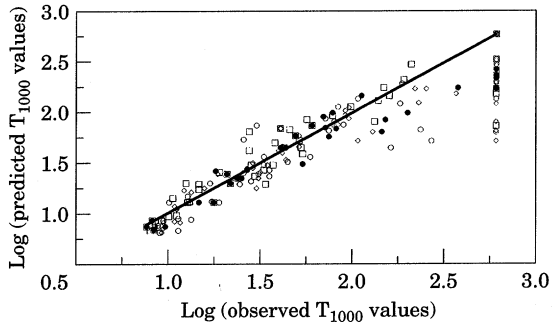

Figure 4. Comparison of T_{1000} values observed with cultures where the water activity of brain heart infusion was modified using mannitol (□), sorbitol (◇), sucrose (○), and sodium chloride (●) with T_{1000} values predicted by the model of Buchanan and Bagi (1994). Variable combinations where no growth was observed were assigned a value of 600 h.

concentrations of the three solutes to inhibit *E. coli* O157:H7 was sorbitol>mannitol> sucrose (Tables 1–3).

In addition to water activity, the growth kinetics were influenced by temperature and pH. Increasing incubation temperatures from 12–28°C decreased GTs and LPDs. Temperature also influenced the range of pH and a_w conditions tolerated by the micro-organism, with 28°C supporting growth over the greatest range of pH and a_w combinations. The pH of the system had relatively little effect on the micro-organism's growth kinetics within the range 5·5–7·5, but decreasing pH to 4·5 strongly depressed growth rates (Fig. 3).

Discussion

While a_w has proven to be an effective means for describing microbial water relations in foods, there are specific solute effects that alter micro-organisms' responses when different humectants are used to modify the amount of biologically available water. This appears to involve both the effects the different solutes have on the physical characteristics of the environment (e.g. viscosity, oxygen solubility) and the compatibility of the solute with the micro-organism's metabolic systems (i.e. the ability of the cell's enzyme systems to continue to function in the presence of high concentrations of the solute) (Ballesteros et al. 1993). It is interesting to note that there was little difference in the micro-organism's response to mannitol and sorbitol even though it does not readily metabolize the latter sugar alcohol. As was expected, solute identity had relatively little impact on the influence of a_w on the growth kinetics of *E. coli* O157:H7 at low solute concentrations. However, differences among the solutes were noted as the a_w became more restrictive. It should be noted that no attempt was made to correct the a_w calculated from the standard curves for temperature. While a_w is not affected over the temperature range used in the current study when NaCl is used as a humectant, small differences can be expected with other solutes.

The ability of the response surface model of Buchanan and Bagi (1994) to predict the behavior of *E. coli* O157:H7 when different solutes were used was assessed by comparing the observed and predicted T_{1000} values for the different data sets, including that for NaCl (Fig. 4). This was accomplished by converting the a_w values into their corresponding NaCl concentration using the equations described above, and employing the NaCl values to solve the response surface polynomial of Buchanan and Bagi (1994). In this evaluation, variable combinations that yielded a no response were assigned a value

of 600 h, the maximum time the cultures were incubated. For the culture conditions that supported growth, the model provided 'fail-safe' T_{1000} predictions over the range of solute concentrations. The agreement between predictions and observed data was best at the shorter T_{1000} values. At the higher sorbitol, sodium chloride, and sucrose levels, the observed growth rates were less than the predicted values. This included the model predicting growth under cultural conditions where no growth was observed. This reflects the fact that the model was generated using a natural logarithm transformation that excluded the no-growth data, thus making the model conservative at those points. It is possible that the addition of a compatible solute (i.e. betaine) could have eliminated some of the no-growth variable combinations or enhanced growth rates. This has been identified as having a potential impact on accuracy of models of *E. coli* growth in minimal medium (Krist et al. 1996), but this should be of less importance in models based on the growth of the micro-organism in more complex media such as the BHI used in the current study.

The ability of the model to effectively predict the behavior of the micro-organism was assessed further using the accuracy and bias indices proposed by Ross (1996). These statistics were 1·37 and 0·92, 1·53 and 0·72, 1·52 and 0·76 and 1·47 and 0·76 for mannitol, sorbitol, sucrose and sodium chloride, respectively. When the no-growth data were excluded, substantially improved accuracy and bias indices (i.e., closer to 1·00) of 1·25 and 1·06, 1·26 and 0·90, 1·36 and 0·88, and 1·28 and 0·89 were obtained. These statistics indicate that the model's accuracy and bias were similar for the four humectants, and would be expected to provide reasonable predictions for combinations of a_w, pH, and temperature that support the growth of the micro-organism.

References

Ballesteros, S. A., Chirife, J. and Bozzini, J. P. (1993) Specific solute effects on *Staphlococcus aureus* cells subjected to reduced water activity. *Int. J. Food Microbiol.* **20,** 51–66.

Bhaduri, S., Turner-Jones, C. O., Buchanan, R. L. and Phillips, J. G. (1994) Response surface model for the effect of pH, sodium chloride, and sodium nitrite on growth of *Yersinia enterocolitica* at low temperatures. *Int. J. Food Microbiol.* **23,** 333–343.

Bhaduri, S., Buchanan, R. L. and Phillips, J. G. (1995) Expanded response surface model for predicting the effects of temperature, pH, sodium chloride contents and sodium nitrite concentrations on the growth rate of *Yersinia enterocolitica*. *J. Appl. Bacteriol.* **79,** 163–170.

Buchanan, R. L. and Phillips, J. G. (1990) Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration, and atmosphere on the growth of *Listeria monocytogenes*. *J. Food Protect.* **53,** 370–376.

Buchanan, R. L. and Bagi, L. K. (1994) Expansion of response surface models for the growth of *Escherichia coli* O157:H7 to include sodium nitrite as a variable. *Int. J. Food Microbiol.* **23,** 317–332.

Buchanan, R. L. and Klawitter, L. A. (1992) The effect of incubation temperature, initial pH, and sodium chloride on the growth kinetics of *Escherichia coli* O157:H7. *Food Microbiol.* **9,** 185–196.

Buchanan, R. L., Bagi, L. K., Goins, R. V. and Phillips, J. G. (1993) Response surface models for the growth kinetics of *Escherichia coli* O157:H7. *Food Microbiol.* **10,** 303–315.

Buchanan, R. L., Stahl, H. G. and Whiting, R. C. (1989) Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes*. *J. Food Protect.* **52,** 844–851.

Chandler, R. E. and McMeekin, T. A. (1989) Modelling the growth response of *Staphylococcus xylosus* to changes in temperature and glycerol/water activity. *J. Appl. Bacteriol.* **66,** 543–548.

Christian, J. H. B. (1981) Specific solute effects on microbial water relations. In *Water Activity: Influences on Food Quality*. (Eds Rockland, L. B. and Steward, G. F.) pp. 825–854. New York, Academic Press.

Gibson, A. M., Bratchell, N. and Roberts, T. A. (1988) Predicting microbial growth: Growth response of Salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *Int. J. Food Microbiol.* **6,** 155–178.

Krist, K., Ross, T. and McMeekin, T. A. (1995) Effect of betaine on *Escherichia coli*. Abst. 8th *Australian Food Microbiology Conference*, Melborne.

McMeekin, T. A., Chandler, R. E., Doe, R. E., Garland, C. D. and Olley, J. (1987) Model for the combined effect of temperature and salt concentration/water activity on the growth of *Staphylococcus xylosus*. *J. Appl. Bacteriol.* **62,** 543–550.

Miller, A. J. (1992) Combined water activity and solute effects on growth and survival of *Listeria monocytogenes* Scott A. *J. Food Protect.* **55,** 414–418.

Palumbo, S. A., Williams, A. C., Buchanan, R. L. and Phillips, J. G. (1991) Model for the aerobic growth of *Aeromonas hydrophila* K144. *J. Food Protect.* **54,** 429–435.

Palumbo, S. A., Williams, A. C., Buchanan, R. L. and Phillips, J. G. (1992) Model for the anaerobic growth of *Aeromonas hydrophila* K144. *J. Food Protect.* **55,** 555–565.

Quintavalla, S. and Parolari, G. (1993) Effects of temperature, a_w, and pH on the growth of *Bacillus* cells and spores: A response surface methodology study. *Int. J. Food Microbiol.* **19,** 207–216.

Ross, T. (1996) Indices for performance evaluation of predictive models in food microbiology. *J. Appl. Bacteriol.* **81,** 501–508.

Sutherland, J. P. and Bayliss, A. J. (1994) Predictive modelling of growth of *Yersinia enterocolitica*: the effects of temperature, pH, and sodium chloride. *Int. J. Food Microbiol.* **21,** 197–215.

Sutherland, J. P., Bayliss, A. J. and Robert, T. A. (1994) Predictive modelling of growth of *Staphylococcus aureus*: the effects of temperature, pH, and sodium chloride. *Int. J. Food Microbiol.* **21,** 197–215.

Sutherland, J. P., Bayliss, A. J. and Braxton, D. S. (1995) Predictive modelling of growth of *Escherichia coli* O157:H7: The effects of temperature, pH, and sodium chloride. *Int. J. Food Microbiol.* **25,** 29–49.

Zaika, L. L., Phillips, J. G. and Buchanan, R. L. (1992) Model for the aerobic growth of *Shigella flexneri* under various conditions of temperature, pH, sodium chloride and sodium nitrite concentrations. *J. Food Protect.* **55,** 509–513.

Zaika, L. L., Moulden, E., Weimer, L., Phillips, J. G. and Buchanan, R. L. (1994) Model for the combined effects of temperature, initial pH, sodium chloride and sodium nitrite concentrations on the anaerobic growth of *Shigella flexneri*. *Int. J. Food Microbiol.* **23,** 345–358.